DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
Rockville MD 20857

• I-009321-E-0083

APR 2 0 2007

U.S. Fish and Wildlife Service
Aquatic Animal Drug Approval Partnership Program
Attention: David Erdahl, Ph.D.
Branch Chief, AADAP
4050 Bridger Canyon Road
Bozeman, MT 59715

Re: Review of a pivotal effectiveness protocol for chloramine-T [CHLT-07-EFF]

Dear Dr. Erdahl:

We do not concur with the protocol entitled The Efficacy of Chloramine-T to Control Mortality Due to Bacterial Gill Disease or External Columnaris Disease in Cool and Warmwater Fish you submitted on February 13, 2007, and amended on March 28, 2007 (T-0083). You submitted this protocol to the investigational new animal drug (INAD) file I-009321 for chloramine-T. Chloramine-T is proposed for the control of mortality due to bacterial gill disease or external columnaris disease in finfish. We found the protocol unacceptable for the following reasons:

GENERAL COMMENTS:

1. Please clarify the schedule of study events in Section 3.2, including all data to be collected on each day, by specifically stating on which study day each event should occur. An example would be the acclimation period will be from Day A to Day 0, fish will be weighed and measured on Day B, the treatment period will be from Day 0 to Day X, and fish will be collected for examination on Day Y. Creating a schedule of events table may aid in organizing study procedures in a logical way.

2. In Sections 3.2.1, 4.4, and 8.2.2 the randomization procedures for assigning fish to tanks, assigning treatments to tanks, and selecting water samples to analyze for dose verification are discussed. Please provide more detailed descriptions of the randomization processes to be used for these procedures. For example, reference could be made in the body of the protocol to SOP No. MISC 237.0 in Appendix E. In this case, please provide a brief description of how the SOP will be used. Regarding the assignment of treatments to tanks, a separate description of randomization should be written for each possible study design that could be used. These descriptions should take into account the characteristics of their respective study designs. (See Biometrics Comment 1)

3. In Section 5.1.1.4 it is stated that initial body size will be determined at the start of each study. Please provide more specific details which describe the process of estimating weight and length based on the measurements of a fixed number of fish. Also, please include the length-weight relationship table which will be used to estimate weight in the protocol.

4. In Section 5.1.1.5 it is stated that the physiological state of test fish will not be determined. Please explain what is meant by the phrase 'physiological state of test fish'. You should record clinical signs of the disease prior to beginning the study. Documenting these clinical signs could include certain aspects of the physiological state of the test fish, for example difficulty respiring or lethargy.

5. In Sections 5.1.1.5 and 5.2 signs and symptoms of the disease of concern are discussed. Please provide examples of the signs and symptoms of bacterial gill disease and external columnaris disease or, alternatively, a reference can be made in the protocol to appropriate sections of Appendix B for bacterial gill disease and columnaris disease.

6. In Section 5.1.2 it is stated that a sufficient number of test fish will be loaded into each test tank to approximate near production loading levels. It is also stated that the number of fish from the reference population allocated to each test tank will be mathematically calculated. Please be more specific about the number of test fish to be used per experimental unit. This would allow similar numbers of test fish to be used at each facility and provide CVM the opportunity to comment on whether the number of test fish to be used is sufficient to provide statistically valid results. If a specific number of fish (or range) is not able to be defined in the protocol then CVM would recommend scheduling a teleconference so that a recommendation for a minimum number of test fish can be discussed. Also, please provide the calculation(s) used to determine the number of test fish to be used so that all investigators are using the same mathematical techniques.

7. Sections 5.2.2 and 5.4.3 state that a sufficient number of fish will be examined by necropsy to confirm the presence of bacterial gill disease and external columnaris disease. Section 5.12 states that 5 to 10 fish from the reference population will be examined for disease diagnosis prior to entering fish into the study. Please be more specific about the number of fish to be examined to establish the disease diagnosis.

   It is stated in Section 5.2.2 that the number of fish to be considered sufficient will be based on the experience of the fish health expert, however, a specific number (or range) could be decided upon before the initiation of the study. This way each investigator will know how many positive diagnoses of disease are required to initiate the effectiveness trial. Also, this will provide uniformity between the facilities so, for example, one facility does not make the diagnosis of disease based on the sampling of 2 fish while another facility bases their diagnosis of disease based on the sampling of 20 fish. Methods to arrive at a more uniform procedure for disease diagnosis can be discussed with CVM in a teleconference prior to submission of a revised protocol.

8. Section 5.3 discusses the exclusion criteria. The information in this section is vague. For events 2 and 3, please qualify "unduly stressed" so that all investigators will exclude experimental units using the same criteria. For event 4, please qualify how many fish

would need to jump out of the tanks or be preyed upon to be considered unacceptable. And if this number is not a specific number (since different numbers of fish will be used per tank at different facilities), then please state what percentage would need to escape from the experimental unit to be considered unacceptable. Please provide more clarification as to the events of the exclusion criteria.

9. Section 5.4.3 is titled 'Baseline data collected prior to initiating the study'. Please include any other data in addition to mortality that will be recorded prior to initiating the study, such as water quality parameters, stocking density, etc.

10. In Section 5.4.3 it is stated that daily mortality in the rearing unit(s) holding the reference population will be collected for at least 3 to 5 days before allocating fish to test units. Please indicate where this information will be captured (e.g., a separate data capture form or as part of the daily facility records) and that this information will be reported in the FSR.

11. In Section 5.5 it is stated that non-blinded personnel may possibly code chloramine-T and sham water treatment containers in such a manner that blinded personnel can dose tanks without knowing which tanks received chloramine-T and which tanks did not. Please describe in more detail this coding technique. This would ensure that all non-blinded personnel are using the same technique at all facilities.

12. In Section 5.12 it is stated that moribund fish from the reference population and test tanks will be sampled periodically during the study to confirm the presence of the pathogen of concern. Disease re-confirmation is not necessary during the treatment period and the removal of moribund fish from study tanks could confound the results of the mortality counts, if they are not appropriately recorded as mortalities.

    However, the collection and examination of recent mortalities [after they have been recorded on the appropriate data capture forms] may be helpful to explain an unexpected rise in mortality. Please revise your protocol to clearly state that moribund fish removed from study tanks will be counted as mortalities. If you choose to examine fish for disease confirmation during the treatment or post-treatment period, the protocol should specify under what situations fish will be examined and where the results of the examination will be recorded. If an unexpected rise in mortality is attributed to another etiologic agent, additional sampling may become necessary.

13. In Section 5.12 it is stated that necropsies will be performed by trained study personnel following guidelines outlined in references such as the Fish Health Section BLUE BOOK 2005 included in Appendix B. There is not a section describing necropsy techniques. Please provide a reference for fish necropsy techniques and include that as either a citation in the reference section or within the appendices. Also, please state that information showing the training of all personnel performing the necropsies will be included in the FSR.

14. In Section 6.1.1 it is stated that mortalities will be colleted, counted, and recorded at least once daily. Please revise the protocol so that mortality is evaluated at least twice daily.

15. In Section 6.1.4.1 the description of the procedure for sample counting is stated. This description is vague. Please provide more details when describing this procedure including an appropriate size for the subsample and how individual weights are determined from weights of a subsample of fish. If there is a standard equation that is used to determine this information please provide that in the protocol. Please provide more specific details when describing this method.

16. Other data to be collected (i.e., water temperature, dissolved oxygen, water hardness, pH, and alkalinity) are discussed in Section 6.2. Please provide accepted ranges for each of these parameters for both cool- and warmwater fish species in either an SOP or as an appendix. Also, some general guidance about procedures to correct the levels for each parameter if the levels drop outside the reference range would be helpful so that steps to mitigate a water quality problem are not taken which might compromise the study.

17. Form 1 entitled 'Test Site, Species Tested, Environmental and Culture Conditions, and Water Quality Parameters' does not appear to have a section for recording water quality parameters, instead this data is recorded on Forms 3 and 4. Please retitle Form 1 appropriately.

18. Mortality data will be captured on Form 2 in Appendix C. This form is designed to capture the data from all days of the trial on the same page. Having all of this data on the same form could interfere with blinding by allowing blinded personnel to see a pattern in mortality numbers over the course of the study and therefore infer which experimental units had been treated and which experimental units were controls. Therefore, please revise this form so that data from each day is recorded on an individual form.

    Also, both Form 2 and Form 3 are currently organized for studies in which the treatments are given every other day. By having a separate data capture form for each day of the study, this would allow investigators to treat once daily or every other day for three treatments without having to correct or rewrite the provided forms.

19. While the protocol does sufficiently address the issue of adverse reactions in Section 6.3, the protocol does not describe how investigators should respond if an adverse event occurs. Adverse events may include excessive mortality, power outages, pump failures, water heater failures, and loss of water, to describe a few. The protocol should provide instructions for investigators to record all observations, mitigations, and results of an adverse event, as well as whom will be contacted if an adverse event occurs. Please revise the protocol with respect to this comment.

20. In Section 8.2.2 it is stated that in the event that results from the dose verification of the one treated sample is greater than $\pm$ 25%, then all samples collected on that day will be analyzed. Please state that the range refers to $\pm$ 25% ***of the intended dose.***

21. In Section 8.2, the procedures for collecting and analyzing samples for dose verification are discussed. According to the title page for the general instructions in Appendix F, it is stated that "at present we recommend completing the analysis as quickly as possible after chloramine-T is added to treatment water. Under no circumstances should the time exceed one-half hours." CVM also agreed with an immediate analysis in their letter

dated July 7, 1997 (I-004168-P-0015). Based on this information, please revise the sample collection and evaluation timeframes so that the samples are collected and analyzed within 30 minutes after the beginning of treatment. If this timeframe is not feasible then please provide an explanation describing the discrepancy between the instructions in Appendix F and those in the body of the protocol.

22. In Section 12.3 it is stated that if possible, protocol deviations will be documented fully when they occur. Please remove the clause 'if possible' as all deviations should be fully documented and explained.

BIOMETRICS COMMENTS:

1. Regarding the study design, if location within the facility is not expected to have an impact on fish mortality or well being, CVM recommends using a completely randomized design. If location within the facility is expected to impact fish well being, we recommend using a randomized block design, where the blocks are designed so that tanks within a block are in a similar environment. When deciding what design to use, we recommend that you contact CVM to discuss the matter.

2. Rather than analyzing the arcsine-square root-transformed mortality rates, CVM recommends using a generalized linear model to analyze mortality. This kind of model takes into account the binomial nature of the incidence of mortality and will improve your power to detect a difference between the mortality rates of the two treatment groups. This analysis can be done, for example, in SAS Proc GLIMMIX. The model should include treatment as a fixed effect. Other effects related to the study design, such as the random effect block for a randomized block design, should also be included when present.

3. Because the different studies conducted under this protocol will vary according to design, species, and disease, CVM recommends doing a separate analysis for each study.

4. A tank should be excluded from the analysis if it is removed from the study for reasons unrelated to BGD or EC or to treatment. These reasons should be documented in the FSR. If you have questions about potential exclusions you may contact CVM prior to your analysis.

ADDITIONAL COMMENTS:

We offer the following recommendations for revision of your protocol. While not required for concurrence of this protocol, we believe that incorporating these recommendations will improve the quality of this study protocol and future protocol submissions.

1. In Section 1.1 you list the causative agent of bacterial gill disease as *Flavobacteria spp.* and the causative agent of external columnaris disease as *Flavobacteria columnare.* The currently accepted genus name for both of these bacteria is *Flavobacterium.* We recommend that you revise the protocol with the most current genus and species name.

Form 2 and Form 3 are currently numbered so that Day 1 is the acclimation day with treatment beginning on Day 2. We recommend that you modify this so that the acclimation day is labeled as Day -1 and the first day of treatment is labeled as Day 0.

2. A list of potential species to be tested has been proved in Section 3.3. Based on the INAD records for chloramine-T the following list of required pivotal and supportive effectiveness studies has been determined:

Temperature Range	**# Pivotal Studies**	**# Supportive Studies**	**Species**
Bacterial Gill Disease			
Coolwater	1	1	Two different coolwater species.
Warmwater	1	1	Two different warmwater species.
External Columnaris Disease			
Coolwater	0	1	A coolwater species other than walleye.
Warmwater	1	1	Two different warmwater species.

3. You may use this information in determining the type of studies and the best representatives from each species group to complete the EFFECTIVENESS technical sections for cool- and warmwater species of freshwater-reared finfish.

4. In Section 5.1.1 the protocol states that the species of test fish used will be characterized as representative cold-, cool-, or warmwater finfish. Because this protocol is meant to evaluate the effectiveness of chloramine-T in cool- and warmwater species, please remove the reference to coldwater species when describing the animals to be tested.

5. CVM notes that your power to detect a treatment effect also improves as the number of tanks increases. If your calculations indicate that you do not have sufficient power, you should consider adding more tanks and/or, if possible, more fish per tank. Please contact CVM if you have any questions or concerns about power.

We recommend that you submit a revised protocol for our review to obtain our concurrence before you begin this study. Our concurrence with your protocol would mean we fundamentally agree with the design, execution, and analyses proposed in your protocol, and that we commit that we will not later alter our perspectives on these issues unless public or animal health concerns appear that we did not recognize at the time of the protocol assessment. However, even with our concurrence, we could make no commitment that the data obtained from a study implementing your protocol will support an approval.

If you submit correspondence relating to this letter, your correspondence should reference the date and the principal submission identifier(s) found at the top of this letter. If you have any questions or comments, please contact me at 301-827-7571 or Dr. Donald Prater, Leader, Aquaculture Drugs Team, at 301-827-7567.

Sincerely,

for

Joan C. Gotthardt, D.V.M.
Director, Division of Therapeutic
Drugs for Food Animals
Office of New Animal Drug Evaluation
Center for Veterinary Medicine